Appendixes

APPENDIX A

Recommendations of the Kinship and Data Analysis Panel (KADAP) to the Office of the Chief Medical Examiner of New York City During the World Trade Center DNA Identification Effort

The Kinship and Data Analysis Panel (KADAP), assembled to assist the Office of the Chief Medical Examiner of the city of New York (OCME) during the World Trade Center (WTC) DNA identification effort, prepared the following recommendations to help the OCME laboratory create policies and procedures specific to the WTC mass fatality incident. These recommendations provided a roadmap when it was necessary to depart from the laboratory's usual forensic casework protocols. The KADAP's recommendations also offered guidance for securing additional resources and provided assurance that sufficient peer review and expertise were available to support these new endeavors.

These recommendations appear here in their original form, without editing. *The annotations in italics offer an after-the-fact context for particular recommendations to the OCME. The KADAP's recommendations are included as appendix A to this report because of their historical significance, and because they may be helpful to laboratories that are developing a mass fatality incident DNA identification response plan. The recommendations and opinions represent a consensus of the KADAP members (referred to in the recommendations as "the Panel") who were present on the date indicated; not all members were present at every meeting.*

1st KADAP (October 18–20, 2001)

The following recommendation sought to inform officials beyond the OCME, New York City Police Department (NYPD), and New York State Police (NYSP) that deviations from protocols would be ongoing, that the local scientists were respected experts in their fields, and that the KADAP was involved in reviewing new protocol developments.

- The Panel recognizes the unprecedented complexity of identifying the victims from the World Trade Center attacks. They also recognize the expertise of the OCME, the New York City Police Department (NYPD), and the NYSP. Given the evolving nature of this task, the Panel stresses that these are their initial recommendations, and they may be modified by OCME, NYPD, or NYSP, as they deem necessary. The panel remains available to them for consultation upon request.

The use of multiple software programs presented numerous difficulties that had to be overcome in the face of the informatics needs of the WTC DNA identification effort and the absence of existing software programs to address the issues. The following recommendations were developed after the KADAP considered the features of all available software programs.

- No single program currently exists that meets all of the analytical needs for resolution of the WTC victims. Therefore, we recommend for the short term:
  - WTC CODIS [Combined DNA Index System] be used:
    - — At high stringency for direct matches. Likelihood ratio of 1 X 1010 is sufficient to report identity. A 13-locus match using the core CODIS loci is sufficient to report identity.
    - — At low stringency to screen for potential first-degree relatives (parent/offspring and some sibs) in order to manually search case-specific data for cases with additional potential relatives.

  - DNA•VIEW be used to assess the putative relationship. A minimum Probability of Relationship of 99.9% is sufficient to report identity by kinship analysis. The minimum prior probability is 1/5000, which can be increased to reflect case-specific issues (e.g., members of service).

- Commercially available pedigree programs should be incorporated for kinship review.
- Middleware should immediately be developed to facilitate use of existing programs.
- A customized program, developed in a modular manner following the proposed process flow, is needed. This package should be designed to analyze complex relationships in a way that integrates validated systems when possible. By October 26, 2001, the mechanism needed to commit resources to this program will be identified and established by NIJ [and reported back] to Inspector Mark Dale.

Because mitochondrial DNA mtDNA testing had received significant public attention in several forensic cases, stakeholder expectations for its use in the WTC response were high, and the OCME laboratory received many inquiries from officials regarding its use on the WTC samples. The KADAP was concerned that this early focus on mtDNA would dilute the effort to yield sufficient short tandem repeat (STR) loci in what were likely to be difficult samples. The Panel was concerned that this might hinder the identification process by adding less powerful methods of identification before all efforts to reveal unique identities had been exhausted.

- Mitochondrial DNA typing of victim samples should be used only as a last resort after additional test reanalysis and/or the use of additional forensically validated STR, Y-chromosome, or other nuclear markers have been used.
- If forensically validated systems, including mitochondrial data, are insufficient to resolve identity, research grade systems should be explored on a case-by-case basis.
- Mitochondrial DNA typing should be performed on all maternal lineage relative's appropriate samples (e.g., buccal swabs, blood) using a suitable validated system on the extracts as provided by NYSP, Myriad Genetics, or any other authorized agency.
- Mitochondrial DNA typing should not be performed on personal effect samples until other appropriate approaches have been considered.

These consensus recommendations represent a major step towards evaluating the complex data that will be generated from the World Trade Center terrorist attacks.

2nd KADAP (November 20, 2001)

With many competing agencies involved in the WTC effort, the KADAP offered recommendations about DNA-specific resource needs to reinforce their urgency with officials in charge of prioritization.

- This Panel determines that it is critical to the success of the WTC identification project that the OCME and NYSP share rapid access to the same data sets via immediate installation of a T1 line.
- The Panel recognizes that requests for prioritization of analyses of particular samples have significant implications for the overall process. Such requests will impede the overall progress of identification, increase the chances of analytical or interpretive errors, and increase costs. The Panel strongly urges those who make such requests to take all of these factors into account and minimize requests for prioritization.

The confirmation of identification by DNA was relied upon by the Chief Medical Examiner. The following recommendation aided in establishing baseline identity estimates.

- The Panel has recommended that likelihood ratios equal to or in excess of 1010 can be adopted as sufficient evidence of identity. However, this value should not be considered as a necessary criterion for identification in all cases, and that final recommendation of identification can properly be based on lower values depending on all available information, as determined by the Chief Medical Examiner.

3rd KADAP (February 21–22, 2002)

The following recommendations considered and addressed sample processing issues. The complexity of the process is shown in the graph

that appears on the last page of this appendix, "WTC Disaster Manhattan (DM) Identification Process."

Production:

- The Panel believes that collaboration and information sharing between the different groups and agencies involved in the DNA identification of the WTC victims is a critical component to maximum identification throughput.
- Numerous production choke points exist as obstacles in meeting the goal of maximum identification throughput. Information management and software integration are major issues that need to be supported to avoid obstacles. The existing software programs should continue to be supported and effective software integration should be developed with appropriate priorities. This requires additional resources, including but not limited to hardware, software, expert systems, and personnel.
- In order to eliminate the most immediate choke points, the Panel recommends that:
  - OCME and NYSP each hire/contract two (2) additional information technology FTEs so that present staff experienced in the current process can be solely dedicated to the WTC effort.
  - OCME and NYSP each hire/contract five (5) additional forensic analyst FTEs to be solely dedicated to the WTC effort.

Validation and Quality Control:

- Documented validation protocols should be developed and implemented for software programs and interfaces.
- Dedicated personnel and equipment should be made available for validation.
- Objective unbiased peer review is a useful process to implement valid systems.
- Appropriate test genetic data should be integrated into the WTC CODIS for efficient validation of all software.
- The current procedures to confirm matches (see attached flow chart) used by OCME and NYSP are appropriate.
- The probability of miscalling alleles that would lead to false inclusions is so small that it is not necessary to review electropherograms previously reviewed by vendor laboratories for uncomplicated STR cases that meet previous recommendations for likelihood ratios.

Continued Testing:

- Successful DNA typing of all samples will not be possible due to conditions of the remains. The Panel recommends that testing of individual samples should be finite. Criteria for determining cessation of testing should be established. Development of a probative test should be investigated.

4th KADAP (April 24–25, 2002)

As the scope of the WTC effort evolved, and the complexities of data management and the number of partnerships increased, the KADAP recommended and implemented a mechanism to facilitate secure, rapid transfer of data and provided additional development of statistical approaches to kinship analyses.

Recommendations:

- In order to facilitate data flow, the Panel recommends that a mechanism of data synchronization should be created. NCBI [National Center for Biotechnology Information] should host the secure FTP resource. The Forensic Biology Unit of the OCME needs Internet access with adequate bandwidth and tools for secure access.
- Cases involving difficult kin interpretations, including such things as mutations, should be reviewed by members of the AABB Parentage Testing Community to recommend disposition to OCME.
- Kinship used to confirm a personal effect match should be accepted at a Probability of Relationship of 99.9% using a Prior Probability of 0.5.

In addition to making recommendations, the KADAP offered several statements to support the work of the OCME and the NYSP.

Statements:

- KADAP recognizes the desire of victims' relatives, public officials, and the concerned

public for complete and accurate use of validated forensic methods for identification of those lost in the WTC attack.

- KADAP recognizes that elected officials and the public must balance the above goals with desire for expeditious reporting of results. These are competing goals which must be considered carefully.
- KADAP recognizes that ongoing scientific and administrative review of all data will be needed to assure the accuracy of victim identifications. KADAP has concerns that imposed time deadlines are not in the best interest of making accurate or complete identifications.
- KADAP fully supports and endorses the efforts to date of the NYC OCME and NYSP in the processing of DNA from victims, personal effects and family members. To date, over 900 identifications have been accomplished using a combination of traditional methods and modern DNA technology.
- KADAP also recognizes that many victims may not be identified despite great effort by all concerned. Similarly, incomplete DNA results on highly degraded samples are likely to preclude positive identification of many of the 19,000 remains from victims recovered to date.
- KADAP is fully committed to ongoing efforts to assist New York agencies in identification of victims and remains. KADAP recognizes that successful DNA typing of all samples will not be possible due to the condition of the remains.
- KADAP recommends that DNA testing of individual samples cannot continue indefinitely (i.e., beyond the limits of sample integrity and available technology).
- Statistical criteria should be reviewed and revised as appropriate for use in assignment of identity of remains yielding incomplete DNA profiles.

The following recommendation was made because results were obtained from fewer loci from later samples recovered from Ground Zero. At the same time, the estimate of the number of victims became more firm, allowing statistical approaches similar to that of a "closed "system to be considered.

Identification Rules:

- Compromised DM samples can be considered associated with samples that were previously matched through DNA if the LR of shared loci [is] >108. This is equivalent to one divided by the random match probability of the shared loci between the two profiles.

5th KADAP (July 15–16, 2002)

As data from fewer loci were recovered from more compromised samples, experimental methods were evaluated for application in the WTC effort. The following recommendations considered parameters for using single nucleotide polymorphism (SNP) methodology in this environment.

Commentary and Recommendations on Use of Linked SNPs for Forensic Kinship Analysis of WTC Samples:

1) Use of the CODIS STR loci is a well-established method for estimation of random match probability and for kinship studies.
2) Unlike the 13 CODIS STR loci, which are unlinked, the 70 SNP loci studied in the KADAP pilot project consist of multiple haplogroups. Many of these SNPs are closely linked with each other and with the CODIS STR loci.
3) While linkage of genetic markers, per se, may have no untoward effect on their use in match probability estimates, linkage between SNPs will alter the calculations used in certain kinship estimates.
4) Use of inherited SNPs is very promising as an adjunct or substitute for STR profiling. A KADAP subcommittee on SNPs met on 12 July 2002 in Washington, D.C. This subcommittee recognized the potential of the technique pending additional studies.
5) KADAP recommends that the OCME of NYC proceed with the pilot use of the ORCHID/Genescreen (Dallas, TX) SNP panels on WTC samples in appropriate situations.
6) Sample consumption issues must be appropriately addressed before SNP analysis proceeds.
7) KADAP also recommends the KADAP SNP subcommittee pursue further statistical analysis of existing SNP data.

6th KADAP (September 9–10, 2002)

As time passed, a more precise list of victims was established. The KADAP reassessed the character of the WTC site a year after the attack and the statistical approaches that could be used.

KADAP Recommendations Regarding Identification of WTC Victims Based on DNA Profiling:

1) For purposes of statistical analysis of genetic data, KADAP recommends that the OCME consider the WTC as a closed population at this time.
2) The size of the closed population is considered to be the number of persons reported missing (currently 2,802).
3) Therefore, KADAP recommends that prior probabilities used in match estimates be based on either the number of:

   (a) RM [reported missing] and the gender ratio, OR

   (b) nongenetically identified RM individuals (of appropriate gender) plus the number of genetically identified individuals who cannot be excluded from the DNA profile in question.

Operationally, KADAP recommends that the OCME use 3(a) above until such time as 3(b) is necessary to refine statistical estimates.

Based on the assumption of a closed population of WTC victims and on the reduced estimate of the number of missing persons (from 5,000 to 2,802), KADAP recommends reducing the threshold for direct matching of remains from a likelihood of 1x1010 to 4x109.

Based on the gender ratio of the Reported Missing WTC victims (as of 9/10/02), the appropriate thresholds for direct matching of remains of known gender are 2x108 for females and 2x109 for males.

MtDNA Recommendations:

- KADAP recommends use of an mtDNA database that reflects, as closely as possible, the population mix of the WTC victims. The mtDNA from one maternal relative or positively identified personal item can serve as the reference sample for the RM. Certain relatives, including spouses, can be used to constitute the mtDNA database. Thus, when multiple relatives of a victim are available, mtDNA profiles from different maternal lineages can be included.
- KADAP recommends that the upper bound of the frequency estimate of an observed mtDNA sequence in a population should, at this time, be reported as:

  $$X/N + 1.96 \div (p(1-p)/N),$$

  where $p = X/N$, and where

  X = # of "matching" mtDNA sequences in a database of size N.

  If X = 0, then the upper bound of the frequency estimate = 1 – alpha(1/N) , where alpha = 0.05

Additional recommendations were made as the SNP technology was assessed.

SNP Recommendation (December 2002)

Based on the UHT [ultra-high throughput] SNP validation data provided by Orchid Biosciences in Dallas, Texas, the KADAP recommends that this technology may be used by the OCME for WTC specimens as a potentially useful, but research grade, identification technology. The KADAP recommends going forward with limited testing of WTC specimens for investigational purposes, proceeding in a staged approach, with continuous evaluation of the utility and validity of this technology.

7th KADAP (January 21–22, 2003)

As the identification effort progressed, review of collection issues highlighted the need to adopt new methods of data collection for future mass fatality situations. The following recommendations were made after dialogues with those responsible for data collection from the Disaster Mortuary Operational Response Teams (DMORT).

KADAP Recommendations to DMORT

The KADAP recognizes the importance of the Victim Identification Program (VIP) as a vehicle for collecting the critical data relied upon for making precise identifications in mass fatality incidents. The VIP can be made more useful to DNA Laboratories by including additional genetic information. Therefore, KADAP respectfully offers the following recommendations:

1) Amend the VIP form to include more comprehensive fields to assist in DNA-based identifications. The KADAP would be pleased to assist the DMORT committee in revision of existing forms.

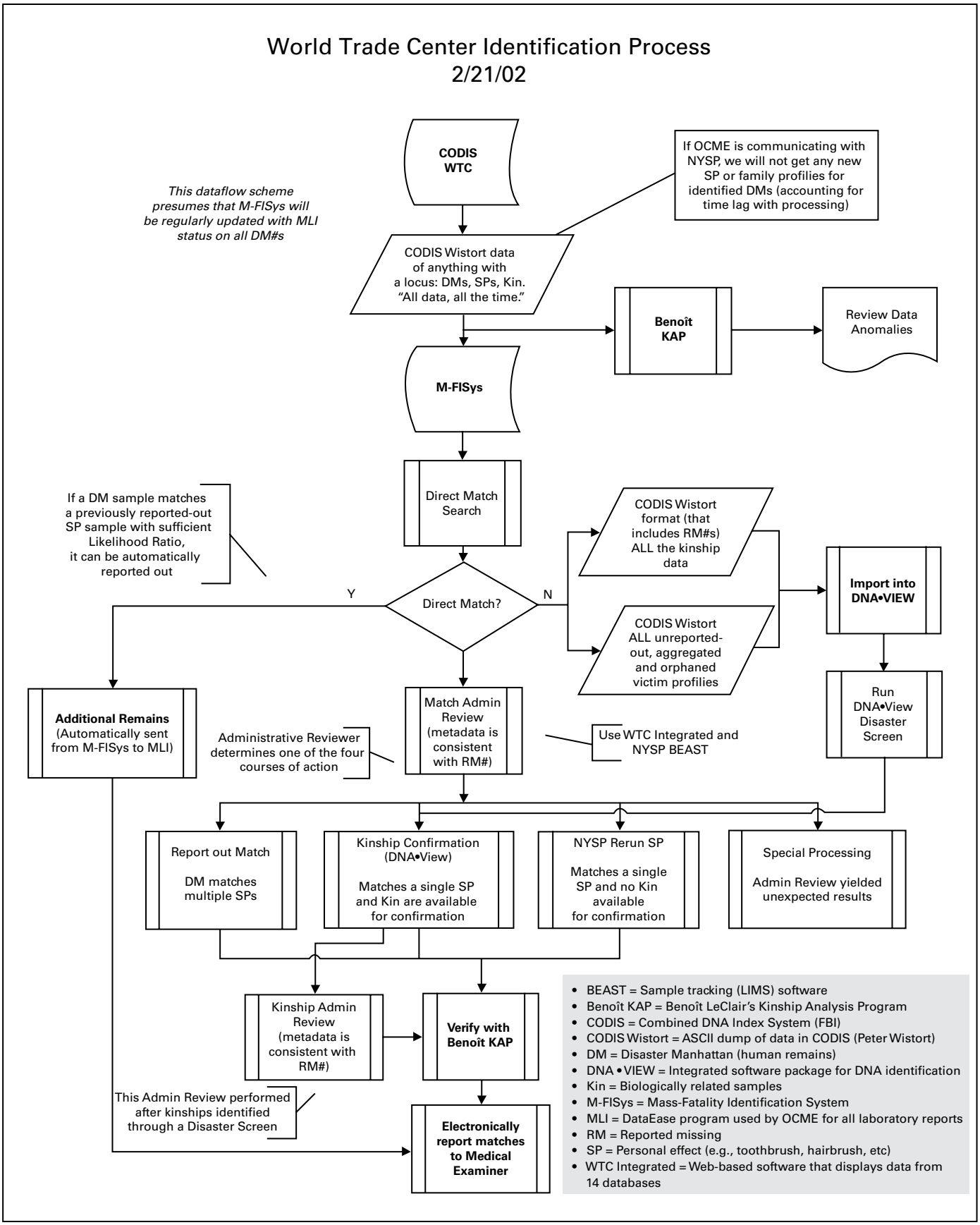
2) DMORT should consider adding one or more DNA identification specialists to the Family Assistance Center (FAC) teams to allow for timely onsite collection of kinship data and personal effects needed for DNA extraction/profiling.

8th KADAP (July 7–8, 2003)

Nearly 2 years after the attack, the KADAP assessed the capabilities of existing technologies for the remaining and most challenging samples. This recommendation was made to help families and other stakeholders understand the limitations of existing technologies for identifying these remains.

KADAP recognizes that DNA testing will not be successful for many samples and therefore some of the WTC victims will not be positively identified by STR, mtDNA or SNP testing.

KADAP further recognizes that OCME has exhausted appropriate contemporary methods of DNA extraction and genotyping on recovered WTC biological samples. While it cannot be ruled out that future scientific advances may reopen promise for additional testing, KADAP recommends that completion of ongoing work with current technologies be viewed as a stopping point in the identification process.


World Trade Center Identification Process
2/21/02
This dataflow scheme presumes that M-FISys will be regularly updated with MLI status on all DM#s
CODIS WTC
If OCME is communicating with NYSP, we will not get any new SP or family profiles for identified DMs (accounting for time lag with processing)
CODIS Wistort data of anything with a locus: DMs, SPs, Kin. "All data, all the time."
Benoît KAP
Review Data Anomalies
M-FISys
Direct Match Search
If a DM sample matches a previously reported-out SP sample with sufficient Likelihood Ratio, it can be automatically reported out
Direct Match?
Y
N
CODIS Wistort format (that includes RM#s) ALL the kinship data
CODIS Wistort ALL unreported-out, aggregated and orphaned victim profiles
Import into DNA•VIEW
Run DNA•View Disaster Screen
Additional Remains (Automatically sent from M-FISys to MLI)
Match Admin Review (metadata is consistent with RM#)
Administrative Reviewer determines one of the four courses of action
Use WTC Integrated and NYSP BEAST
Report out Match
DM matches multiple SPs
Kinship Confirmation (DNA•View)
Matches a single SP and Kin are available for confirmation
NYSP Rerun SP
Matches a single SP and no Kin available for confirmation
Special Processing
Admin Review yielded unexpected results
Kinship Admin Review (metadata is consistent with RM#)
Verify with Benoît KAP
This Admin Review performed after kinships identified through a Disaster Screen
Electronically report matches to Medical Examiner
BEAST = Sample tracking (LIMS) software
Benoît KAP = Benoît LeClair's Kinship Analysis Program
CODIS = Combined DNA Index System (FBI)
CODIS Wistort = ASCII dump of data in CODIS (Peter Wistort)
DM = Disaster Manhattan (human remains)
DNA • VIEW = Integrated software package for DNA identification
Kin = Biologically related samples
M-FISys = Mass-Fatality Identification System
MLI = DataEase program used by OCME for all laboratory reports
RM = Reported missing
SP = Personal effect (e.g., toothbrush, hairbrush, etc)
WTC Integrated = Web-based software that displays data from 14 databases

APPENDIX B

Sample Personal Items Submission Form

After a mass fatality incident, friends and family members will provide authorities with personal items that may contain a missing person's DNA. The DNA profile obtained from the personal item(s) will be searched against the profiles obtained from the remains samples. To efficiently and effectively use DNA analysis to identify human remains, it is important that personal items be correctly identified.

The purpose of this sample form is to help a laboratory:

- Determine who is missing.
- Provide information on the types of personal items that loved ones should submit.
- Identify the submitter and the items being submitted.
- Clarify what other DNA might be on the item; for example, if personal items of the missing individual are submitted, a reference sample from a spouse, domestic partner, or full-time roommate may be useful, even if no biological relationship exists.
- Begin chain-of-custody documentation for the items.
- Obtain permission from the submitter to test the items.
- Provide notification that the articles may be damaged or destroyed during testing.

Personal Items Submission Form

Missing Individual Information

Last Name	Suffix (Jr., Sr.)	First Name	Middle Name	Sex (circle) M F

The missing person is/has been known by the following additional names (include maiden name)	Date of Birth Year: _ _ _ _ Month: _ _ Day : _ _	Social Security Number _ _ _ -_ _-_ _ _ _

Submitter Information

Last Name	Suffix (Jr., Sr.)	First Name	Middle Name

Telephone numbers (in order of preference)

1st : ( ) 2nd: ( ) 3rd: ( )

Home Street Address	City	State

Country	ZIP Code	E-mail address

I am providing a reference sample from the missing individual.
I am the missing individual's_____.
(e.g., mother, father, sister, son, roommate)

Please list the personal items below:

Item Number	Item Description	Other Possible DNA Sources on Item. Please Explain.
0	*Example: Pink toothbrush with white handle*	*My husband and I may have used the same toothbrush*
1		
2		
3		
4		
5		
6		

Name of Missing Individual:_____
(Last, First, Middle, Suffix)

Please note:

- *If there is a possibility that there may be someone else's DNA on a personal item, it is helpful to submit a biological sample from the person(s) who might have also used the item (reference sample). Please refer to the* ***Sample Family and/or Donor Reference Collection Form****.*
- *Items submitted should be directly attributable to the missing individual.*
  - *Biological samples suitable for testing include:*
    - *Bloodstain cards (e.g., newborn screening cards [Guthrie cards] or cards obtained from other repositories).*
    - *Oral swabs (e.g., from home DNA identification kits).*
    - *Blood stored for elective surgery.*
    - *Pathology samples (e.g., biopsy samples, PAP smears).*
    - *Extracted teeth (baby/wisdom).*
    - *Hair samples.*
  - *Personal items that might contain the missing individual's DNA include:*
    - *Used toothbrushes.*
    - *Used shavers/razors.*
    - *Unwashed undergarments and other suitable clothing items.*
    - *Used personal hygiene items (e.g., feminine sanitary napkins).*
    - *Other personally handled or used items (consult the testing laboratory for specific criteria).*

I, _____ hereby grant permission to
(Please print or type name of submitter)
extract and type DNA from the items listed on page 1 for the purpose of assisting in the identification of a missing person. I understand that in the testing process the item may become damaged or destroyed and may not be returned.

_____ _____
(Signature of submitter) *(Date)*

The items were received on _____at _____
(Date) *(Collection location)*

_____
(Collection address)

Sample(s) received by _____ _____.
(For testing agency use only)

APPENDIX C

Sample Family and/or Donor Reference Collection Form

After a mass fatality incident, a missing person's friends and family members provide identification information to officials who are handling the recovery and identification efforts. Complicated family structures — for example, multiple marriages, adoptions, same-sex partners — present challenges in collecting family relationship information. Obtaining an accurate family structure helps minimize gaps in information.

The information requested in this sample form is quite comprehensive, including a description of the jewelry worn by the missing individual, dental history, and a list of family members who may be able to provide DNA samples for the kinship identification process. This information typically is stored in the Victim Identification Program (VIP), a database supplied by the Federal Emergency Management Agency (FEMA). VIP is the central repository of all missing individual identification information, which can be accessed by pathologists, laboratory personnel, and medical examiners who are involved in the identification process.

Once family members have been identified and documented in the VIP, DNA samples need to be collected. Collection kits — used to collect the family and donor reference samples to determine biological relationships — should be available at family assistance centers and can be sent to family members all over the world.

The purpose of this sample form is to assist the laboratory in:

- Determining the identity of the missing individual.
- Identifying the donor of the reference sample.
- Clarifying the biological relationship between the missing individual and the donor; for example, if personal items from the missing individual are being submitted for analysis, a reference sample from a spouse, domestic partner, or full-time roommate is useful even if no biological relationship exists.
- Obtaining chain-of-custody information for the family reference sample.
- Obtaining permission to test the sample.
- Providing information on the best types of family reference samples to collect.

Family and/or Donor Reference Collection Form

(Each donor needs to fill in a separate form and submit a separate sample for each missing person.)

Missing Individual Information

Last Name	Suffix (Jr., Sr.)	First Name	Middle Name	Sex (circle) M F

The missing person has been known by the following additional names (include maiden name)	Date of Birth Year __ __ __ __ Month __ __ Day __ __	Social Security Number or citizenship (if not a U.S. citizen) __ __ __ - __ __ - __ __ __ __

Donor Information

Last Name	Suffix (Jr., Sr.)	First Name	Middle Name

Telephone numbers (in order of preference)

1st :( ) 2nd :( ) 3rd :( )

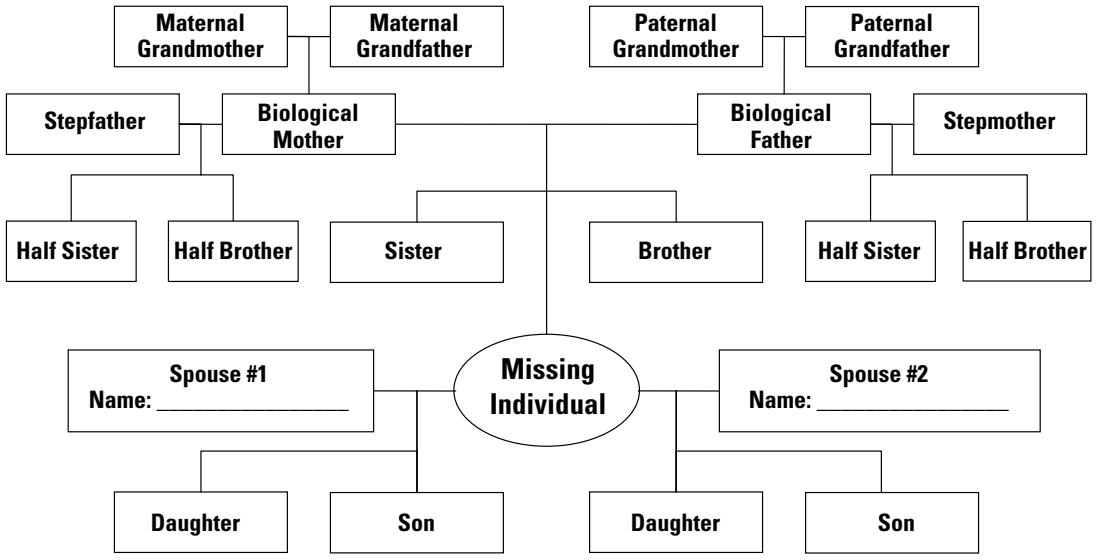
Home Street Address

City	State	ZIP	Country

Date of Birth Year __ __ __ __ Month __ __ Day __ __	Sex (circle) M F	E-mail address (please print)

I am providing a family reference sample, as I am the missing individual's _____
(e.g., mother, father, sister, son)

Please circle your relationship to the missing individual:



Other: *(please specify)* _____ *(e.g., grandchild, friend, roommate)*

Name of Missing Individual:_____
(Last, First, Middle, Suffix)

Please note:

- *If personal items of the missing individual are being submitted for analysis, a biological reference sample from the spouse, domestic partner, or full-time roommate is useful even if no biological relationship exists. Please refer to the* ***Personal Items Submission Form*** *when submitting personal items.*
- *The biological parents and biological children are the best comparison samples for identification through kinship. If these samples are unavailable, samples from other biological relatives may be submitted.*
- *If a child provides a sample for parental identification, the child's other biological parent should also provide a sample.*
- *For identification through kinship analysis:*
  - *Full siblings are preferable over half siblings.*
  - *Grandparents should provide a sample only if the mother or father cannot provide a sample.*
  - *Grandchildren should provide a sample only if their parent, who is related to the missing individual (as a son or daughter), is unavailable.*
- *The laboratory will assess the samples provided. The most appropriate sample(s) will be used to identify the missing individual. The family may be contacted if additional samples are needed.*

I am also a relative of the following other missing individuals:_____

I, _____ hereby grant permission to extract and type
(Please print or type name of donor)

my DNA for the purpose of assisting in the identification of a missing person.

_____ _____
(Signature of donor or guardian if donor is a minor) *(Date)*

The sample was collected on _____ at _____
(Date) *(Collection location)*

_____
(Collection address)

Sample was collected by *(if self-collected indicate "self")*_____

APPENDIX D

Sample Family Tree Form

The complexity of modern family structures (e.g., multiple marriages, adoptions, same-sex partners) can challenge the collection of family relationship information. The purpose of this Sample Family Tree Form is to help a laboratory:

- Determine who is missing.
- Identify the individual providing the information.
- Provide family relationship information.

This type of form should be completed each time someone provides information about a missing individual and/or donates a sample. Because of the complexity of determining biological relationships, it generally is advisable to have a trained interviewer — such as a geneticist or genetic counselor — complete the form.

FAMILY RELATIONSHIP TREE

Victim's Name:_____
(Last, First, Middle, Suffix)

It is important for the DNA laboratory to correctly understand the victim's family structure when using DNA to establish biological relationships and identify victims. In order to obtain an accurate family structure, please complete the contact information for the interviewee (the person providing the information on the family relationships) as well as your contact information as the interviewer. This form may be completed each time a new individual provides information about a potential victim and/or donates a sample using the family reference collection form. Using the directions below, please describe the family of the victim, including the interviewee, the victim, and all other close relatives.

Interviewee Contact information:

Last Name:	Suffix:	First Name:	Middle Name:
Telephone numbers (in order of preference): 1st: ( ) 2nd: ( ) 3rd: ( )			
The interviewee is the missing individual's_____ *(e.g., mother, sister, son, roommate)*			

Interviewer Information:

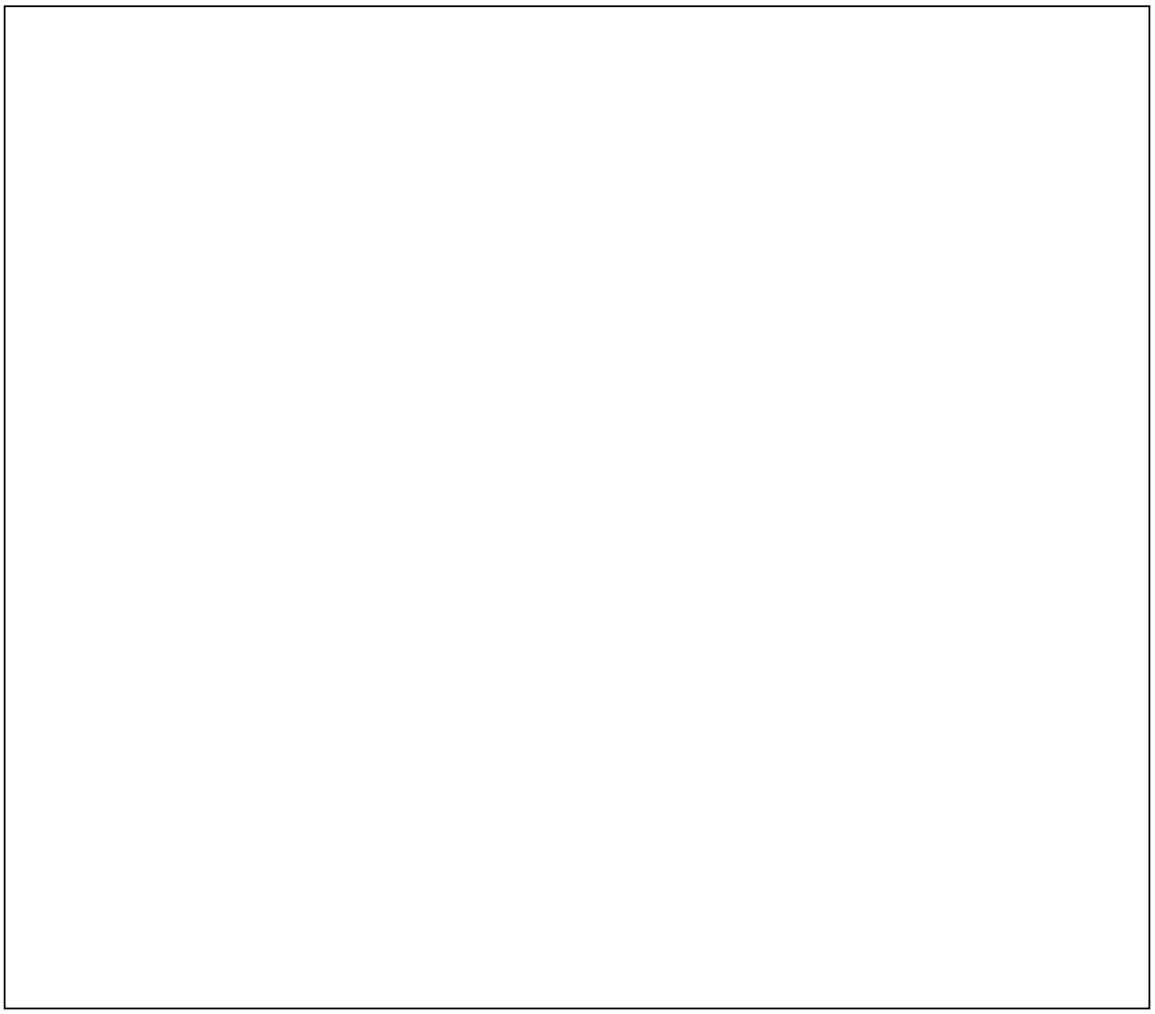
Name:	Date:
Affiliation and address:	
Telephone numbers (in order of preference): 1st: ( ) 2nd: ( ) 3rd: ( )	

Directions:

- Use the box on the other side of the page to draw the family tree.
- A picture of the family should be drawn by placing the interviewee in the center, providing he or she is biologically related to the missing individual.
- Use circles for women and squares for men.
- Put each person's name in the circle or square.
- In the circle or square, indicate whether the individual is living, deceased, or missing.
- Draw a line between parents and place children below the line.
- Include wives and husbands.
- Provide a narrative if you think it will be helpful.
- If the interviewee is not biologically related to the victim, indicate his or her relationship to the victim and draw the victim's family structure as outlined above.
- Add comments below the box to clarify relationships as needed.

Victim's Name:_____
(Last, First, Middle, Suffix)

Family Tree:



Comments:_____

_____

_____

_____

_____

_____

_____

_____

APPENDIX E

Guidelines for Family and/or Donor Reference Collection Kit Components and Oral Swab Collection Instructions

To obtain a properly collected and labeled sample, it is preferable to use a tamper-evident, presealed oral swab collection kit. Some laboratories may prefer to have the swabs air-dry for 15 minutes to an hour prior to placing the oral swab in the swab envelope. Although the process of air-drying the swabs may lead to a more pristine sample, the process of air-drying is risky and may inadvertently lead to a sample mixup if more than one person's sample is collected at a time. The laboratory may also want to incorporate some type of notification system in which the collection location calls or faxes the DNA laboratory when the sample has been collected, alerting the DNA laboratory that the sample is on the way. A tamper-evident, presealed oral swab collection kit may contain:

Collection instructions (See sample Oral Swab Collection Instructions below)

Collection form for family reference sample (See Sample Family and/or Donor Reference Collection Form, appendix C)

Form describing the family relationship (See Sample Family Tree form, appendix D)

Pair of gloves (preferably one-size-fits-all Nitrile gloves)

Sterile, cotton-tipped swabs (2–6)

If the collection is performed correctly on a healthy individual, two swabs are sufficient to get adequate amounts of DNA for a short tandem repeat (STR) analysis. If extended testing may be required, it is preferable to collect six swabs.

Fastener (optional)

A small rubber band or twist-tie may be included to bind together all of the swabs from one individual prior to placing them in the swab envelope. Alternatively, a label may be included to secure and label the swabs.

Swab envelope

Once the swabs have been collected, they should be placed in an envelope that can be uniquely identified with the donor's information.

Tamper-evident, sealable bag, containing desiccant packet

If mass collections are to be performed, inadvertent sample switches may occur if the swabs are allowed to air-dry in the open; therefore, a desiccant can be used to help keep the moist swab from molding. If a Ziploc bag is used, tamper-evident police-evidence seals can be placed on the bag.

Mailing envelope

A preprinted mailing envelope with an appropriate prepaid shipping label will help ensure that the swabs are delivered to the correct location. Make sure the shipping carrier services the area where the sample will be collected. Different air bills and customs documents may be needed if samples will be shipped from outside the United States.

Oral Swab Collection Instructions

To avoid sample mixups, identification, collection, and sample sealing should be performed for *one* individual at a time. Also, it would be advisable to:

- Have a trained individual interview the family member and complete a family tree.
- Wear gloves while collecting the sample, and change gloves before collecting from the next individual.
- Collect samples from one individual at a time.
- Verify the identity of the individual whose sample is being collected and confirm that the mouth is free of tobacco products, gum, food, etc., before collecting the oral swab. If necessary, have the individual rinse his or her mouth with water prior to collection.

- Have the donor fill out a Family and/or Donor Reference Collection form.
- Open the swab packages provided, being careful to not handle the cotton tip of the swabs.
- Remove one swab and collect the specimen by rubbing the swab vigorously and thoroughly on the inside surfaces of the cheeks and gums. Rub the swab up and down and back and forth about 10 times, while slowly turning the swab, so that all sides of the swab are in contact with the side of the cheek.
- Place the swab in the envelope provided. Do not place the swab back into the original packaging. Repeat the process with the remaining swabs.
- Identify the swab envelope with the date, the donor's name, and the collector's name. Have the donor sign the envelope to verify the information.
- Complete the collection information on a Family and/or Donor Reference Collection form, and verify that the donor completed the requested information.
- Seal the swab envelope. Place the swab envelope and completed Family and/or Donor Reference Collection form in the plastic bag with the desiccant, and place in shipping envelope. Maintain the sample in a cool, dry environment until shipment. Do not store under extreme hot or cold conditions.

APPENDIX F

Issues to Consider When Outsourcing Reference Samples

There are many issues a laboratory director must consider when making the decision to send mass fatality samples to an outside vendor for short tandem repeat (STR) analysis testing. This list of issues is not meant to be inclusive; rather, it is offered as a starting point to aid in considering the use of a vendor laboratory to test personal items, reference samples, or remains samples.

Tasks and Requirements

- What standards of quality assurance are to be met.
- What certification will be provided that testing is performed in accordance with quality assurance standards.
- Specific tasks (for example: "The Vendor shall analyze all samples for the 13 CODIS core STR loci plus Amelogenin — FGA, vWA, D3S1358, CSF1PO, TPOX, THO1, D18S51, D21S11, D8S1179, D7S820, D13S317, D5S818, and D16S539 — in accordance with the Federal Bureau of Investigation's NDIS [National Data Index System] Standards for Acceptance of DNA Data and the Contracting Agency/Vendor Testing and Reporting Guide.").
- Accreditations/certifications that the vendor laboratory should maintain, and penalties if accreditation/certification is not maintained.
- Timeframe for analysis and reporting turnaround (for example, "x" kinship samples per week, etc.).
- External proficiency testing program(s) that the vendor must complete during a specific timeframe, along with terms for submitting a certified statement of compliance and documentation of any failed proficiency tests and the remediation that was done to resolve the issue(s).
- Terms regarding the individual DNA analyst's compliance with a semiannual external proficiency testing program.
- Requirements that changes in the vendor's key personnel (specific personnel) be approved.
- Protocols and procedures for making analysis of the samples, quality control documents, and validation documentation available for review, inspection, and monitoring, including onsite reviews of the vendor's facility and records.
- Standard operating procedures and quality assurance procedures (including any changes made during the process) with respect to the receipt and analysis of samples.
- Terms regarding the vendor's ability to subcontract (or prohibition against subcontracting) any portion of the testing or analysis of the samples to any other laboratory without prior written authorization.
- Format for processing samples (for example, "Whole blood in tubes that the vendor shall be required to stain onto cotton fabric, 903 S&S paper, FTA paper," etc.; buccal swabs on a swab or placed on 903 S&S paper or FTA paper; extracted DNA; personal items (toothbrushes, hair brushes, clothing); victim bone and tissue, etc.).
- Preprinted shipping labels and shipping containers, and requirements regarding notification of when a shipping container is received, including notification upon discovery of any damage to the shipping container that would compromise the integrity of a sample.
- Chain-of-custody documentation, including, for example, a unique identifier on the overnight shipping label, sample receipt (and verification of seal integrity), sample transfers during processing, analysis and reporting, and return of the samples and resulting data.
- Storage of samples.
- Use of automated transfers (for example, use of a "plate fingerprinting" system to uniquely identify a 96-well plate, including the strategic placement of known controls on a 96-well

plate in a manner that allows any plate mixup to be detected).

- Use of NDIS-approved STR analysis kits specified in the NDIS Standards for Acceptance of DNA Data; if applicable, use of NDIS-approved STR analysis platforms and expert systems.
- Analytical procedures (for example, using appropriate controls and standards on each gel/run/batch; each sample used in reporting having an acceptable extraction positive, extraction negative, amplification positive, amplification negative, and ladder associated with each locus, and, if a sample is rerun, all controls to be rerun).
- The manner in which data are to be reported (for example, genotypes to be compiled in the common message format for insertion into the FBI's Combined DNA Index System (CODIS) and transmitted in electronic form (floppy disk, CD-ROM, a ZIP disk, secure Web site, or other method); cost of CD-ROM or ZIP disks and shipping to be included in the proposed cost per sample of completed analysis).
- Return of extraction, amplification, gel data sheets (including spreadsheets, original gel scans, and the final gray-scale/color-corrected gel images), and electropherogram data; return of instrument data collection files and files generated in the analysis of the samples in a prescribed form (CD-ROM, ZIP disk, posted to a secure Web site, etc.); return of samples, DNA extracts, amplified product, etc.
- Determination of when the analysis of a specimen is considered complete (for example, not until genotypes for all 13 CODIS core STR loci (plus Amelogenin) have been generated and accepted; requirements for when a sample does not yield a complete profile (for example, retest the sample a minimum of two times, altering conditions within the boundaries of the laboratory's written standard operating procedures, as necessary, to produce a complete profile, etc.).
- Terms for analysis failure (requests for additional samples, etc.).
- Sample shipping responsibilities (method, chain-of-custody safeguards, timeliness, tracking, etc.).
- Confidentiality of samples and the results of testing, including handling outside inquiries.
- Ownership of data, materials, and documentation.
- Procedures for notification regarding problems in testing.
- Contamination quality assurance checks.
- Retention of testing and quality control records.
- Written weekly reports, including changes to management and key personnel; assessment of technical risks and analytical and quality control processes; description of analytical errors detected during processing and corrective action taken; customer service logs; and performance metrics by sample type (reference, disaster, personal items), including, for example:
  - Number of samples received.
  - Running total for samples received.
  - Number of samples reported.
  - Number of failed samples (for example, those in which no profile or an incomplete profile — not all 13 CODIS core loci + Amelogenin — was generated.
  - Number of samples received more than 30 days ago, but not yet tested, analyzed, and reported.
  - Biweekly briefings.

Deliverables and Delivery Schedule

- Testing, analysis, and reporting services, including shipping; DNA profile; quality control results and records; testing and chain-of-custody documentation; data generated during the receipt, testing, analysis, and reporting; and unused samples.

Suspension and Termination

- Terms for suspension or termination for poor performance, including quality issues, customer service complaints, and inability to meet sample throughput commitments.

Equipment and Materials

- Who will furnish equipment and materials.

Security, Place of Performance, and Period of Performance

Here is a sample vendor testing and reporting guide that may contain components that laboratory directors may consider when contracting with an outside vendor.

(One form for each sample type: family reference, disaster, personal item)

Sample Type_____

1. Samples will be provided to the vendor in the following manner:

2. Samples will come from the following agencies/locations:

3. Samples will be provided to the vendor at the rate of:

4. Samples will be provided with the following identification, which shall be reported with the profile:

5. Samples will be rejected by the vendor for testing for the following reasons, with the following course of action:

6. No more than ___ percent of a sample shall be consumed by the vendor without permission.

7. DNA shall be extracted to a final volume of _____ at a concentration of _____.

8. The following DNA aliquots shall be made for additional testing:

9. The vendor shall use only the following testing and analysis systems:

   Extraction method:

   Amplification conditions (including kit and amplification volume):

   Analysis platform:

   Conditions for retesting if a complete profile is not initially obtained:

10. Procedural changes affecting sample processing must be approved ___ days prior to the processing of samples.

11. Manual transfer shall be allowed only during the following steps:

12. Spiking or enriching a sample is acceptable ___yes ___no.

    Comments:

13. Vendor controls:

    a. Amplification positive

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    b. Amplification negative

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    c. Extraction positive

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    d. Extraction negative

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

    Other:

       Name:
       When introduced:
       Considered acceptable when:
       Location on analysis:
       Location in data files:
       Acceptable results:

A data file is defined as _____
_____.

14. Samples with the following microvariants do not need to be retested:

15. Samples with trialleles shall be processed in the following manner:

16. Samples with triallelic profiles ___shall ___do not need to be retested. The following documentation shall be reported:

17. Samples with microvariants (not on an approved list) ___shall ___do not need to be retested. The following documentation shall be reported:

18. Profiles exhibiting multiple contributors shall be handled in the following manner:

19. Data analysis:

    a. General peak characteristics

       The following reporting criteria apply to:

       _____ Samples

       _____ Ladders

       _____ Controls

       _____ Internal size standard

       Minimum peak height:
       Maximum peak height:
       Shape:

       Spikes ___not allowed ___allowed under the following circumstances:

    b. Internal size standard

       The following peaks are required to be present for reported samples:

       Size of 245 peak (on 310) must be _____.

    c. Allelic Peaks

       Stutter:

       –A:

       Minimum allowable peak height ratio:

20. Data reporting

    a. Composite profiles (instances where the 13 CODIS core loci are created from more than the minimum multiplex data file[s] because one or more of the loci do not meet reporting criteria) ___shall ___ shall not be acceptable unless:

    b. Nonreported samples ___ may ___shall not be intermixed in reported data files.

    c. Data from all sample runs ___must ___ need not be provided.

    d. Minimum and maximum number of reportable samples with complete profiles in a single data file is:

    e. Minimum and maximum number of samples (complete 13-locus profile) in a reported batch:

    f. The following documentation shall be provided/associated with the reported profiles:

    g. Data and data files shall be reported in the following format:

    h. Data shall be reported at a frequency of:

21. Samples shall be returned on the following date and in the following condition:

22. Other:

APPENDIX G

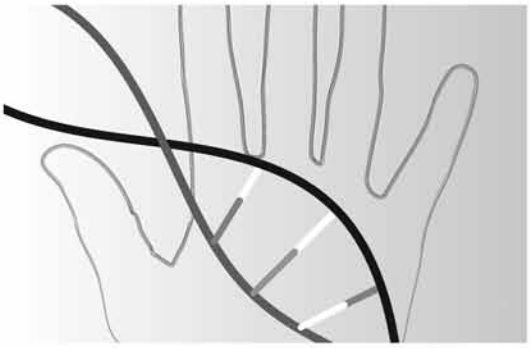
Identifying Victims Using DNA: A Guide for Families

This is a PDF file of a publication (English/Spanish) that can be downloaded at http://www.ojp.usdoj.gov/nij/pubs-sum/209493.htm; to order hard copies, call 1–800–851–3420 or order online at www.ncjrs.gov.

April 2005

Identifying Victims Using DNA: A Guide for Families

In English and Spanish



PRESIDENT'S DNA INITIATIVE

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The National Institute of Justice is the research, development, and evaluation agency of the U.S. Department of Justice. NIJ's mission is to advance scientific research, development, and evaluation to enhance the administration of justice and public safety. This and other publications and products of the U.S. Department of Justice, Office of Justice Programs, and NIJ can be found at *http://www.ojp.usdoj.gov/nij*.

The National Human Genome Research Institute (NHGRI) is one of 27 institutes and centers at the National Institutes of Health, an agency of the Department of Health and Human Services. NHGRI supports grants for research, training, and career development at sites nationwide and conducts research on its campus to develop and implement technology to understand, diagnose, and treat genomic and genetic diseases. Information about NHGRI can be found at *http://www.genome.gov*.

April 2005	
	Identifying Victims Using DNA: A Guide for Families
	NCJ 212872

What is this brochure about?

Any circumstance in which lives are lost is a tragedy that can have immediate and lasting effects on our communities. We extend our most sincere condolences and sympathy to you at this difficult time. You have been given this brochure to help you understand the process of identifying the remains of a victim through DNA analysis.

Why go through the process of identifying remains?

The decision to pursue identification of the remains of a victim through DNA testing is very personal and may be different for each family. Some families may find comfort in knowing that the remains of their loved one have been identified and returned. These remains can be interred according to the family's traditions. This may help with the healing and adjustment to their terrible loss. For others, the testing process may interfere with their healing.

For DNA testing to work, it may be necessary to gather more information, samples, or personal items. Gathering these may cause your family further distress. If the testing does not identify your loved one's remains, it may be a disappointment, adding to your grief.

DNA testing can be provided to help those families who want it. If you choose not to, your decision will be honored. You may take time to talk about it with others who you feel are appropriate. People who can help include family, friends, religious leaders, health professionals, and victim advocates.

1

How is this testing done?

In many cases, DNA testing is one of the best methods to identify a victim or victims. DNA is the material in cells that stores the inherited traits that make up our bodies. In many (but not all) cases, DNA can be isolated from human remains or other samples. To identify the remains of a victim, DNA from remains must be matched to DNA known to be from the victim or the victim's relatives. Thus, it is necessary to collect DNA samples from family members and from personal items or prior medical specimens from the victim.

How long will the process take?

The process of identifying a victim might be relatively quick or it can be quite lengthy. In some instances, not every victim can be identified. When an identification is made, the next of kin will be notified and asked if they wish to be contacted if more remains are found in the future.

How can I help identify my loved one?

Accurate and complete information about the victim (unique physical characteristics, dental records, etc.) should be submitted. Sometimes this information will be sufficient to render an identification. In many cases, such information may have been provided prior to considering DNA testing. To have any success with DNA testing, samples from relatives of the victim will need to be collected to compare with the remains.

2

What are the sources of DNA samples that can be used?

DNA can often be obtained from the biological remains. This DNA will be compared to DNA known to be from the victim or to DNA from the victim's relatives.

What are the sources of DNA from the victim?

DNA from the victim's previously collected medical specimens or personal items can be used to make a direct match to remains. For example, if a loved one recently had surgery or blood work done, a specimen may have been stored at the hospital or clinic. You should provide any known medical specimens or ask for help in locating them. The first row of the table below provides examples of the kinds of medical specimens the laboratory can use.

DNA Sources	Examples	Degree of Usefulness
Medical specimens	Bone marrow donor sample Biopsy sample Newborn screen bloodspot	Most useful
Personal items	Toothbrush Hairbrush	Very useful
Close relatives	Biological parents of victim Children of victim Brother or sister of victim	Useful
Other relatives	Maternal relatives (aunts, uncles, cousins, half-sisters or -brothers on the victim's mother's side)	Less useful

DNA from the victim may also be found on their personal items. The second row of the table above gives some examples of these. A toothbrush or other items containing saliva are often good sources. However, it is very important that these items were used only by the victim or rarely used by anyone else. For example, a hairbrush used by the whole family would not be a good source of DNA from the victim.

How can DNA from relatives be used?

If personal items or medical specimens are not available or if the testing on them does not work, DNA testing can be done on samples from blood relatives. The DNA from adoptive parents, adopted children, stepparents, or other nonblood relatives cannot provide information on the genetic identity of a victim.

The ability to match victims to their relatives depends on how closely related they are to the victim. The most useful DNA samples are from close blood relatives such as the victim's biological mother, father, children, brothers, or sisters. This is because DNA of close relatives is more similar than the DNA of more distant relatives. The pictures on the following pages show the relatives who are most useful for identifying a victim. If DNA from the victim's children is used, it is helpful to have DNA from the children's other biological parent.

DNA from more distant relatives can be used, but this is more difficult. In some cases, samples may be requested from specific relatives. For example, DNA samples could be requested from a maternal relative of the victim such as the victim's aunt, uncle, or half-brothers or half-sisters on the mother's side of the family.

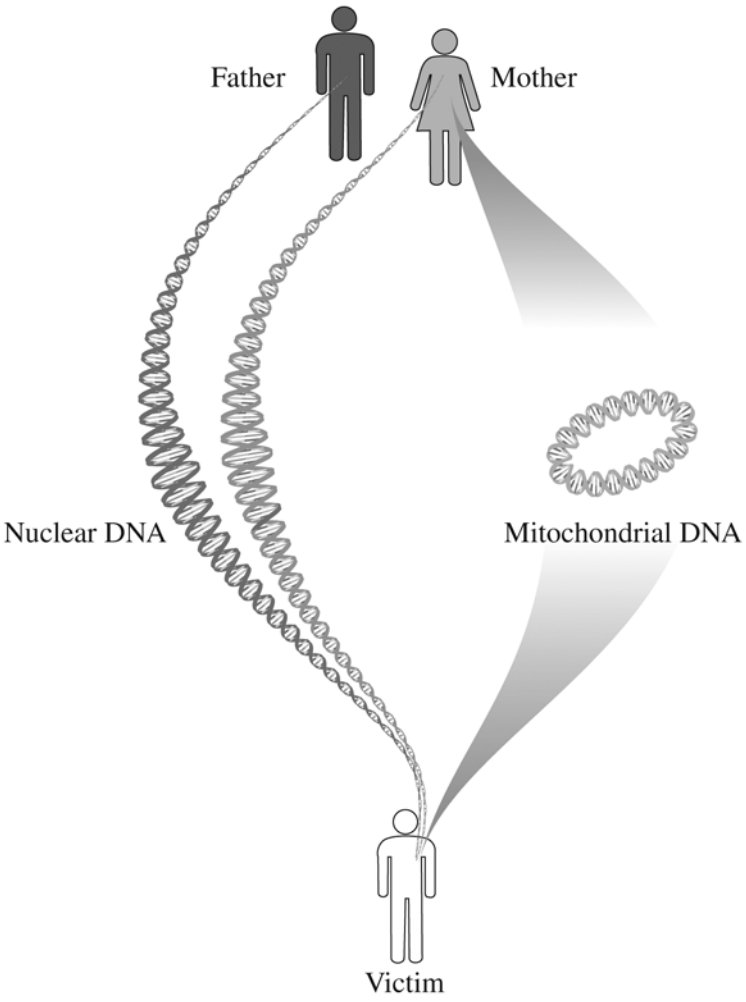
4

Why might DNA analysis not work?

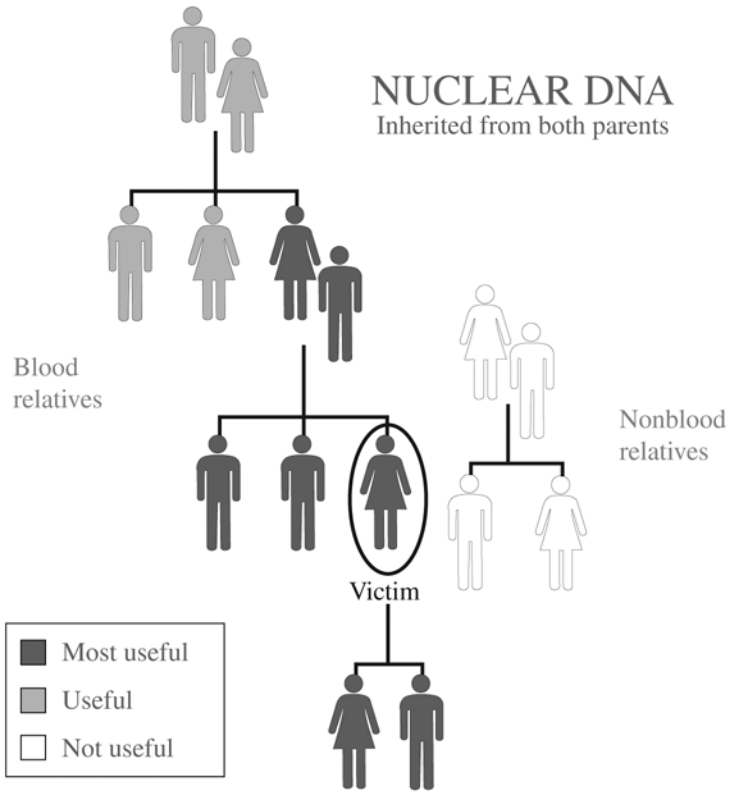
DNA testing might not be able to identify your loved one. The most likely reason would be that there is no usable DNA in the recovered remains. Some victims' remains may not be found. Also, DNA testing may not work if no usable DNA can be found on personal items submitted.

How does DNA testing work?

DNA is the hereditary material that contains instructions to build a human being. DNA can be collected from very small amounts of blood, mouth (cheek) scrapings, hair roots, or other samples. There are two kinds of DNA in the body: nuclear DNA and mitochondrial DNA. Both kinds of DNA can be used for DNA identification.



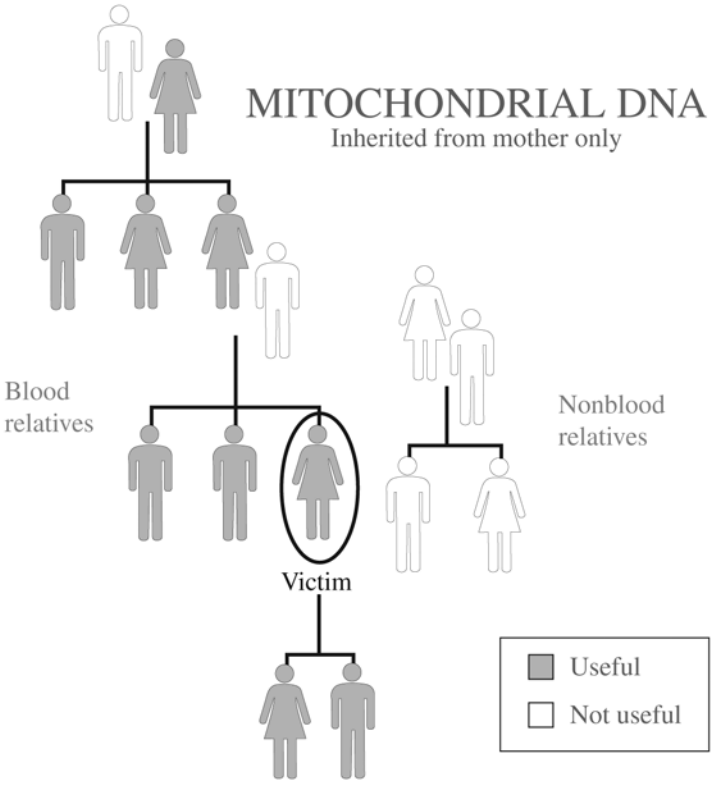
Nuclear DNA comes from the cell nucleus and is inherited from both parents, half from the mother and half from the father (see figure below). Each person's nuclear DNA is unique—except for identical twins, who have the same DNA. When a sufficient nuclear DNA profile from the victim's remains matches the nuclear DNA profile from a sample known to have come from the victim, we can be very sure of the identity of the victim.



Because of the way it is inherited, DNA from blood relatives is somewhat similar. Nuclear DNA from the victim's remains can be compared to nuclear DNA from family members to identify the victim in some circumstances.

The second kind of DNA is called mitochondrial DNA (mtDNA). It is inherited only from the mother (see figure on page 7). Fathers never pass on mitochondrial DNA to

their children. However, mitochondrial DNA typically is not as powerful for making identifications as nuclear DNA. This means that in some instances two unrelated people may have similar mitochondrial DNA. Because of the way it is inherited, only maternal relatives, such as a brother, sister, or mother, can be used for mitochondrial DNA testing.



Nuclear DNA can be easily damaged by extreme heat and other conditions and therefore is not always available to be used for an identification. Mitochondrial DNA, however, can often be found in very small or damaged DNA samples. Typically, scientists test nuclear DNA first. If there are insufficient results for an identification, they will attempt mitochondrial testing. Despite best efforts, some testing may not be successful. But the scientists seeking to identify your loved one will work hard to do so and provide closure for your family.

NIJ is a component of the Office of Justice Programs, which also includes the Bureau of Justice Assistance, the Bureau of Justice Statistics, the Office of Juvenile Justice and Delinquency Prevention, and the Office for Victims of Crime.

8

Findings and conclusions of the research reported here are those of the authors and do not necessarily reflect the official position or policies of the U.S. Department of Justice.

President's DNA Initiative Partners

Office of Justice Programs

National Institute of Justice

Office on Violence Against Women

Bureau of Justice Assistance

Office of Community Oriented Policing Services

Federal Bureau of Investigation

Office for Victims of Crime

Office of Juvenile Justice and Delinquency Prevention

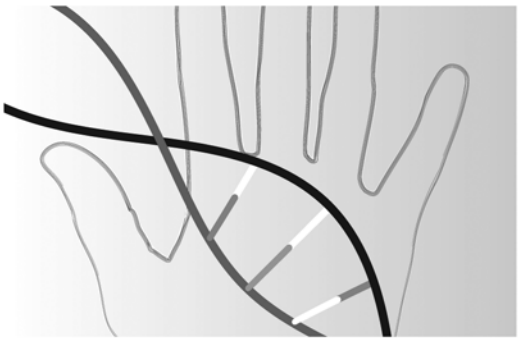
9

NCJ 212872

Abril de 2005

Guía para las familias sobre la identificación de víctimas mediante análisis de ADN

En inglés y español



LA INICIATIVA DEL ADN DEL PRESIDENTE

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Oficina de Programas de Justicia

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Director Interino, Instituto Nacional de Justicia

El Instituto Nacional de Justicia es la agencia de investigación, desarrollo y evaluación del Departamento de Justicia de Estados Unidos. La misión del NIJ es promover la investigación, el desarrollo y la evaluación científica con el fin de fomentar la administración de justicia y la seguridad pública. Puede encontrar ésta y otras publicaciones y productos del Departamento de Justicia de Estados Unidos en *http://www.ojp.usdoj.gov/nij*.

El Instituto Nacional de Investigación del Genoma Humano (NHGRI) es uno entre 27 institutos y centros de los Institutos Nacionales de la Salud, una agencia del Departamento de Salud y Servicios Humanos. El NHGRI ofrece apoyo mediante subvenciones para las labores de investigación, capacitación y desarrollo de carreras profesionales en locales por toda la nación y realiza investigación en sus propias instalaciones para desarrollar e implantar la tecnología orientada a comprender, diagnosticar y tratar enfermedades genómicas y genéticas. Puede encontrar información sobre el NHGRI en *http://www.genome.gov*.

Abril de 2005	
	Guía para las familias sobre la identificación de víctimas mediante análisis de ADN NCJ 212872

¿De qué se trata este folleto?

Toda circunstancia en la que la se pierden vidas es una tragedia que puede causar efectos inmediatos y duraderos en nuestras comunidades. Deseamos expresarle nuestro más sentido pésame durante estos momentos difíciles. Se le ha entregado este folleto para ayudarlo a comprender el proceso para identificar los restos de una víctima mediante el análisis de ADN.

¿Por qué realizar el proceso de identificar los restos de una persona?

La decisión de solicitar la identificación de los restos de una víctima mediante el análisis de ADN es muy personal y puede ser distinta para cada familia. Algunas familias pueden sentir consuelo al saber que los restos de su ser querido han sido identificados y devueltos. Estos restos pueden sepultarse según las tradiciones de la familia. Esta experiencia puede ayudar a las personas a recuperarse y adaptarse a la terrible pérdida. Para otros, el proceso de análisis podría interponerse en la recuperación.

Para que funcione el análisis de ADN, es posible que sea necesario recopilar más información, muestras u objetos personales. Reunir estos objetos podría apenar aún más a la familia. Si la prueba no identifica los restos de su ser querido, podría sentirse desalentado y afligirse aún más.

El análisis de ADN puede ofrecerse para ayudar a las familias que desean que se realice. Si usted elige no solicitarlo, se honrará su decisión. Tal vez desee conversar sobre este tema con algunas personas que considere adecuadas. Las personas que pueden ayudar son, entre otras, la familia, las amistades, los líderes religiosos, los profesionales de la salud y los defensores de víctimas.

1

¿Cómo se realiza esta prueba?

En muchos casos, el análisis de ADN es uno de los mejores métodos para identificar a la víctima o las víctimas. El ADN es el material en las células que contiene los rasgos hereditarios que componen nuestro cuerpo. En muchos casos (pero no en todos), el ADN puede aislarse de los restos de un ser humano o de otras muestras. Para identificar los restos de una víctima, el ADN de los restos debe ser compatible con el ADN que se sabe con certeza que proviene de la víctima o de los parientes de dicha víctima. Por lo tanto, es necesario obtener muestras de ADN de los miembros de la familia y de objetos personales, o bien, muestras médicas previas de la víctima.

¿Cuánto tiempo toma el proceso?

El proceso de identificación de una víctima puede ser relativamente rápido o sumamente prolongado. En algunos casos, no es posible identificar a las víctimas. Cuando se logra la identificación, notificarán al pariente más cercano y le pedirán si desea que se comuniquen con él o ella si se hallan más restos en el futuro.

¿Cómo puedo ayudar para identificar a mi ser querido?

2

Debe presentarse información correcta y completa sobre la víctima (características físicas especiales, registros dentales, etc.). A veces esta información será suficiente para lograr la identificación. En muchos casos, es posible que dicha información ya se haya proporcionado antes de haberse estudiado la posibilidad de realizar un análisis de ADN. Para que el análisis de ADN tenga éxito, es necesario obtener muestras de los parientes de la víctima y compararlas con los restos.

¿Qué fuentes de muestras de ADN pueden usarse?

El ADN con frecuencia puede obtenerse de restos biológicos. Este ADN se compara con el ADN que se sabe con certeza que proviene de la víctima o con el ADN de los parientes de la víctima.

¿Qué fuentes de ADN se usan de la víctima?

Es posible utilizar el ADN de muestras médicas obtenidas previamente o de objetos personales de la víctima para realizar una prueba de compatibilidad directa con los restos. Por ejemplo, si el ser querido tuvo recientemente una cirugía o análisis de sangre de laboratorio, alguna muestra podría haberse guardado en el hospital o clínica. Debe proporcionar cualquier prueba médica que provenga con seguridad de la víctima o pedir ayuda para obtenerla. El primer renglón de la tabla a continuación ofrece algunos ejemplos de los tipos de muestras médicas que puede usar el laboratorio.

Fuentes de ADN	Ejemplos	Grado de utilidad
Muestras médicas	Muestra de un donante de médula ósea Muestra de biopsia Prueba de evaluación con mancha seca de sangre del recién nacido	Más útil
Objetos personales	Cepillo dental Cepillo para el cabello	Muy útil
Parientes cercanos	Padres biológicos de la víctima Hijos de la víctima Hermano o hermana de la víctima	Útil
Otros parientes	Parientes maternos (tías, tíos, primos, hermanastras o hermanastros del lado de la madre de la víctima)	Menos útil

También es posible obtener el ADN proveniente de objetos personales de la víctima. El segundo renglón de la tabla de arriba ofrece algunos ejemplos. Un cepillo dental u otros objetos que contengan saliva a menudo son buenas fuentes de ADN. Sin embargo, es de suma importante que estos objetos los haya usado sólo la víctima o pocas veces otra persona. Por ejemplo, un cepillo para el cabello que usa toda la familia no es una buena fuente de ADN de la víctima.

¿Cómo puede usarse el ADN de los parientes?

Si no hay disponibles objetos personales ni muestras médicas, o si la prueba no funciona en ellos, el análisis de ADN puede realizarse en las muestras de sangre de los parientes. El ADN de padres adoptivos, hijos adoptados, padrastros y madrastras u otros parientes que no tengan consanguinidad con la víctima no ofrecen información sobre la identidad genética de dicha víctima.

La capacidad de emparejar a las víctimas con sus parientes depende del grado de parentesco que tengan los parientes con la víctima. Las muestras más útiles de ADN provienen de parientes consanguíneos cercanos, como la madre, el padre, los hijos, hermanos o las hermanas biológicas de la víctima. Esto se debe a que el ADN de los parientes cercanos se asemeja más que el ADN de parientes distantes. Las imágenes en las siguientes páginas ilustran los parientes que son más útiles a la hora de identificar a una víctima. Si se usa el ADN de los hijos de la víctima, es útil obtener el ADN de los hijos del otro padre biológico.

Es posible usar el ADN de parientes más distantes, pero esta tarea es más difícil. En algunos casos, se podrían solicitar muestras de parientes específicos. Por ejemplo, es posible solicitar muestras de ADN de un pariente por parte de la madre de la víctima, como la tía, el tío o los hermanastros o hermanastras de la víctima del lado de la familia de la madre.

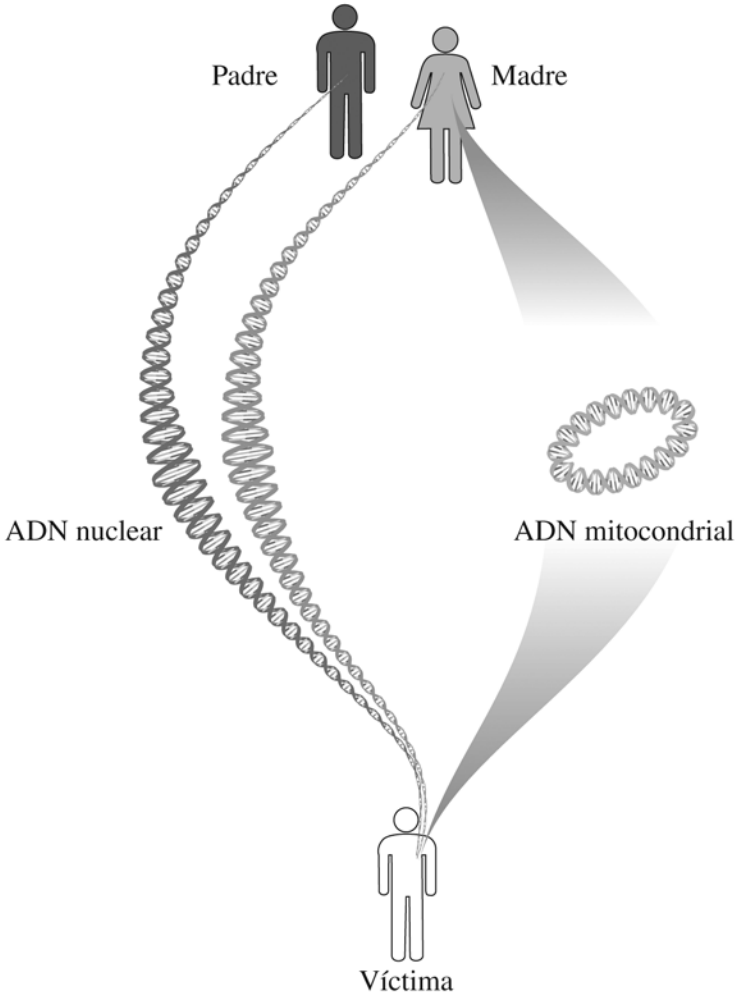
4

¿Por qué a veces no funciona el análisis de ADN?

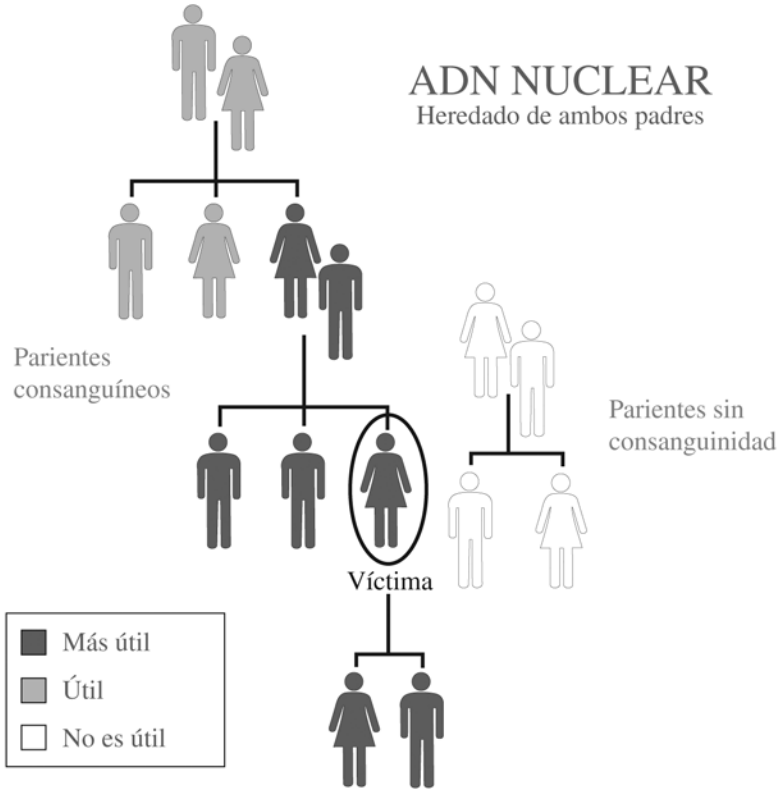
Puede que el análisis de ADN no identifique a su ser querido. La razón más probable de ello es que no hay ADN utilizable en los restos recuperados. A veces tampoco es posible encontrar los restos de algunas víctimas. Además, el análisis de ADN no funcionará si no es posible encontrar ADN utilizable en los objetos personales entregados.

¿Cómo funciona el análisis de ADN?

El ADN es el material hereditario que contiene las instrucciones que forman el ser humano. El ADN puede obtenerse a partir de pequeñas cantidades de sangre, raspaduras de la boca (de la parte interior de las mejillas), raíces del cabello u otras muestras. Hay dos tipos de ADN en el cuerpo: ADN nuclear y ADN mitocondrial. Ambos tipos de ADN pueden usarse para el análisis de identificación por ADN.

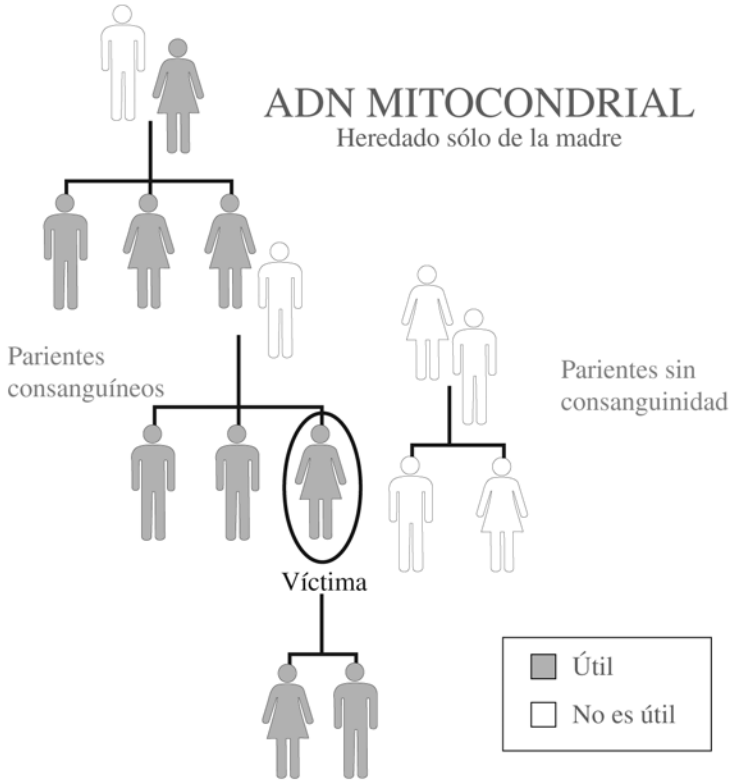


El ADN nuclear proviene del núcleo de las células y se hereda de ambos padres, la mitad de la madre y la mitad del padre (consulte la figura a continuación). Cada persona tiene un ADN nuclear único—excepto los gemelos idénticos, quienes tienen el mismo ADN. Cuando una cantidad adecuada del perfil de ADN nuclear de los restos de la víctima es compatible con el perfil de ADN nuclear de la muestra que se sabe con certeza que proviene de la víctima, podemos identificar a la víctima con mucha seguridad.



Debido a la forma en que se hereda, el ADN de parientes consanguíneos es algo parecido. El ADN nuclear de los restos de la víctima puede compararse con el ADN nuclear de los parientes para identificar a la víctima en algunas circunstancias.

El segundo tipo de ADN se denomina ADN mitocondrial (mtDNA). Este ADN se hereda sólo de la madre (consulte la figura). Los padres varones nunca pasan el ADN mitocondrial a sus hijos. Sin embargo, el ADN mitocondrial por lo general no es tan confiable como el ADN nuclear para la identificación. Esto quiere decir que en algunos casos el ADN mitocondrial de dos personas que no guardan parentesco alguno puede ser semejante. Debido a la forma en que se hereda, sólo los parientes maternos, como un hermano, hermana o madre, pueden usarse para analizar el ADN mitocondrial.



El ADN nuclear puede deteriorarse fácilmente en condiciones de calor extremo y bajo otras circunstancias, por lo tanto, no siempre está disponible para usarse con fines de identificación. El ADN mitocondrial, no obstante, puede encontrarse a menudo en muestras de ADN muy pequeñas o

deterioradas. Por lo general, los científicos prueban primero el ADN nuclear. Si los resultados no son lo suficientemente definidos para la identificación, procurarán entonces analizar el mitocondrial. A pesar de realizar los mejores esfuerzos, puede que algunos análisis no sean exitosos. Pero los científicos que buscan identificar a su ser querido trabajarán arduamente para lograrlo y permitirle a su familia poner fin a esta situación.

El NIJ forma parte de la Oficina de Programas de Justicia, que también incluye el Despacho de Asistencia Jurídica, el Despacho de Estadística Jurídica, la Oficina de Justicia Juvenil y Prevención de la Delincuencia, y la Oficina para Víctimas del Crimen.

Los hallazgos y conclusiones que se expresan en este documento corresponden al autor y no representan necesariamente la posición ni las políticas oficiales del Departamento de Justicia de Estados Unidos.

Colaboradores en la Iniciativa del ADN del Presidente

Oficina de Programas de Justicia

Instituto Nacional de Justicia

Oficina de Violencia Contra la Mujer

Despacho de Asistencia Jurídica

Oficina de Servicios Policiales Orientados a la Comunidad

Oficina Federal de Investigaciones

Oficina de Víctimas del Crimen

Oficina de Justicia Juvenil y Prevención de la Delincuencia

10

APPENDIX H

Sample Analysis: An Overview

While a step-by-step discussion of the processes involved in DNA typing is likely to be too rudimentary for most laboratory directors, it may offer useful information for family assistance coordinators, policymakers, reporters, and others who require a mid-level technical explanation of the issues faced by a forensic laboratory that is responding to a mass fatality incident.

Before a mass fatality incident occurs, laboratories should develop a plan for extraction procedures, alternate analytical methods for challenging samples, automation for handling high-volume analyses, and expert system software to interpret results. One of the critical steps in this process is the creation of a chain of custody documentation system for all materials collected at the scene. This is important not only for scene reconstruction and quality control, but also in the event of any subsequent legal procedure; as in any situation with potential criminal implications, the proper collection and preservation of samples — using the best forensic practices — is critically important. In addition, improper preservation methods can lead to the loss of typable DNA, compromising the ability to make an identification.

Any information that provides reliable identification is valuable. Although this report focuses on DNA analysis, other traditional identification methods (anthropology, dental records, tattoos, etc.) should be used whenever possible, and the metadata should be used in a corroborative way. Some of these identification assays are so uniquely identifying that they may eliminate the need for the more labor-intensive DNA analysis or minimize the need for reanalysis. Furthermore, upfront anthropological screening will be beneficial for identifying the best samples for DNA analysis.

Sample Receipt Accessioning and Storage

Once samples are collected and preserved at the site, they are sent to the laboratory for analysis. The magnitude of samples delivered to the laboratory after a mass fatality incident can be overwhelming. Receiving, accessioning, and storing such samples can disrupt normal laboratory practices because most crime laboratories are not prepared to accommodate such a surge in numbers of samples. To ensure that sample identification is reliable, the laboratory should institute a quality control process to accommodate the surge in sample receipts. If an existing Laboratory Information Management System (LIMS) is not sufficient, one should be created to handle the mass casualty situation. While it is possible that existing chain-of-custody procedures will be sufficient, this issue should be evaluated before a mass fatality incident occurs.

In the event of a mass fatality incident, it is likely — as occurred after the World Trade Center (WTC) attacks — that other laboratories will offer assistance to the lead laboratory. If appropriate chain-of-custody, accessioning, and other infrastructural concerns can be addressed, some of the capacity problems can be shared or outsourced. If samples are sent to other laboratories at any stage of the analysis, the same quality control and chain-of-custody practices must be maintained.

DNA Extraction

The first step in the analytical process is extracting DNA from the reference and disaster samples. Successful DNA typing relies on isolating DNA of sufficient quantity, quality, and purity to yield an adequate DNA profile. DNA extraction protocols that overcome, remove, or dilute enzymatic inhibitors are the most desirable.

The quantity and quality of DNA yielded from a mass fatality sample can be compromised by conditions specific to the event and can range from apparently pristine to highly degraded to substantially contaminated. Disaster samples and personal effects samples may be degraded and contaminated with materials that inhibit analytical processes, particularly for enzymatic reactions such as the polymerase chain reaction (PCR), an in vitro process that increases the amount of small, specific targeted sequences.

Care should be taken to get the best quality DNA possible in order to maximize the number of loci that will be amplified. Consider an extraction procedure that will yield DNA suitable for mitochondrial testing or low copy number (LCN) testing. Also, it is important to keep in mind that it may not be apparent which test systems will be useful until a first round of testing is completed.

The process for DNA extraction is laborious and time consuming. This can be exacerbated in a mass fatality identification if a large number of bone samples — often, the only type of sample available — are sent to the laboratory. Bones can contain substances that inhibit the PCR; therefore, inhibitory substances must be removed if the DNA is to be suitable for typing. In these cases, a laboratory may need to modify its routine extraction procedures to remove PCR inhibitors.

Standard DNA extraction procedures exist for the types of materials that may be encountered. They include: (1) organic solvent, (2) column exchange, and (3) cation exchange resins, such as Chelex–100. The quality of recovered DNA will be limited by the quality of the sample. For some samples, sufficient high-molecular-weight DNA without chemical contaminants may be obtained. For others, the environmental destruction may have been so great that no usable DNA is available for typing. Thus, extraction methods that minimize the loss of DNA are the most desired.

Short Tandem Repeat (STR) Analysis

It is most expedient for laboratories already experienced in DNA casework to use well-known and well-established technologies such as short tandem repeat (STR) typing as their initial method of analysis—and, in fact, many disaster samples may be typable by STR analysis. The 13 core STR loci currently used in the United States and many other countries are composed of tandemly repeated DNA sequences, each of which is typically 4 or 5 base pairs in length. The number of alleles at the forensically employed STR loci typically ranges from 5 to 20.

Amplified STR alleles are manufactured to be somewhat larger, up to 500 bases in length. Because of this, the starting (or template) DNA must be of sufficient quality and quantity to achieve full typing of all the STR loci. When DNA of this quality and quantity is available, STRs can be typed — including with the use of commercial kits that are available to assist in typing the multiple loci (multiplexing) — with a high degree of specificity and sensitivity in a relatively short time period.

Electrophoresis, a process that separates charged molecules in an electric field, is a cornerstone in forensic DNA typing. For the standard forensic loci, the size of the PCR product for an individual is determined by comparison with a commercially available alleleic ladder. To resolve STR loci, most laboratories employ capillary electrophoresis, and the instrumentation associated with this analysis enables automation that allows a higher throughput analysis.

Alternative Testing Methods

In some mass fatality incidents, samples may be so compromised that alternate DNA analysis techniques will be needed to achieve complete identification. The best technologies will, of course, depend on the state of the art, including the ability to demonstrate the reliability of new technologies on compromised samples. Molecular biology is a dynamic field, and new analytical tools are always being developed.

In the WTC response, the Office of the Chief Medical Examiner of New York relied on the recommendations of the Kinship and Data Analysis Panel (KADAP) to help explore new methods to further the identification of compromised samples. For example, the panel looked at whether there would be sufficient extracted material to support all attempted technologies and satisfy quality control inquiries that might arise. The KADAP also considered how to handle statistical

issues using the additional technologies, including linkage and haplotype/genotype comparisons.

Making the Identification

In the WTC identification effort, when the DNA profile from a victim matched a reference sample or was included within a reference family pedigree, statistical significance was placed on the likelihood of such an occurrence. A certain threshold was required for assigning identity. (See appendix A.)

Generally, such a quantitative assessment is based on the frequency of occurrence of alleles from major population groups, such as African-Americans, Asians, Caucasians, and Hispanics. Once the individual frequencies of each independent genetic marker are determined, the frequencies are multiplied using the product rule to estimate the rarity of each of those characteristics occurring as a single profile. It is the combination of the genetic markers that enables the identification.

When personal items are the reference samples, a direct comparison of the profiles is performed, and a random match probability is calculated for those samples that are considered a potential source. For family reconstructions, DNA profiles from relatives are compared with the sample profile (e.g., a mother and a father of a missing child). A likelihood ratio is generated to evaluate whether sufficient evidence exists to support a biological relationship.

A large number of genetic markers are available for identity testing of human remains, and, by typing a sufficient number of these loci, identifications equivalent to uniqueness can be made readily for some, but not all, samples. Limitations include:

- Sample degradation or a sample that is too small to analyze, allowing only a partial DNA profile. This reduces the power to unequivocally identify the source of the sample.

- The existence of reference samples is critical to making an identification. Even if a mass disaster sample yields a complete DNA profile, an identification may not be possible if there are insufficient reference samples. For example,
  it may be relatively easy to identify a missing child when his or her biological parents and two siblings are typed. However, if the only relative available for comparison is a half-sibling, the genetic information will be far more limited and an identification may not be possible. Therefore, every effort should be made to obtain samples from as many close family members as possible. Personal effects enable direct comparisons of profiles, but at times the alleged source of a personal effect is questionable. Obviously, the more that is known about a personal item, the greater the confidence in using it as a reference sample.

- Because of the violent nature of many mass disasters, remains can be commingled. In such cases, a mixture of DNA profiles may be observed. The best practice is to avoid interpreting such profiles; it is better to perform a reextraction from the sample, if possible.

APPENDIX I

Additional References on Statistical Issues in DNA Identification

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